RAPID COMMUNICATION

Inhibition of Interleukin-1 by an Interleukin-1 Receptor Antagonist Prevents Graft-Versus-Host Disease

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Graft-versus-host disease (GVHD) is the major complication of allogeneic bone marrow transplantation (BMT). Dysregulation of inflammatory monokines such as tumor necrosis factor α (TNFα) has been noted in both clinical and experimental GVHD. We present evidence that interleukin-1 (IL-1), another inflammatory monokine, is an important mediator of GVHD. Expression of the gene for IL-1α as well as the gene for TNFα is increased in the skin of mice with GVHD. Inhibition of IL-1 function by the in vivo administration of IL-1 receptor antagonist (IL-1ra) reduces the immunosuppression and mortality of GVHD without impairing the engraftment of hematopoietic stem cells. GVHD thus appears to be a systemic inflammatory process in which monokines, especially IL-1, appear to be important mediators. Inhibition of IL-1 by IL-1ra represents a novel approach to the understanding and control of GVHD.

GRAFT-VERSUS-HOST disease (GVHD) is the primary complication of allogeneic bone marrow transplantation (BMT). Although GVHD is caused by T cells in the donor graft, one manifestation of the disease is the dysregulation of inflammatory monokines such as tumor necrosis factor α (TNFα).[1-3] In an experimental model of GVHD, TNFα has been shown to induce GVHD lesions and inhibition of TNF reduces GVHD in both experimental and clinical BMT.[4-6] Several cytokines are dysregulated in models of in vivo alloreactivity, including graft rejection and GVHD.[7,8] Interleukin-1 (IL-1) is a potent cytokine mediator of a variety of inflammatory conditions[9,10] and has similar effects to TNFα both in vitro and in vivo.[11,12] We wished to examine the role of IL-1 in the pathogenesis of GVHD and asked whether GVHD could be prevented by a molecule that inhibits the action of IL-1.

The human IL-1 receptor antagonist (IL-1ra) is a recombinant protein that, like its natural analog, competes with IL-1 for binding to the type I IL-1 receptor but has no agonist activity.[13,14] IL-1ra has been shown to prevent the effects of exogenous IL-1α in mice,[15] but it has no direct effect on the activity of other monokines such as TNF,[16] (P. Kilian, unpublished results). Thus, this protein is a useful probe of the involvement of IL-1 in the pathology of a complex disease such as GVHD in which multiple cytokines are dysregulated. In this study, we investigated the role of IL-1 in the pathogenesis of GVHD and show that IL-1ra blocks the mortality and immunosuppression associated with GVHD.

MATERIALS AND METHODS

Mice and BMT. CBA and B10.BR mice were obtained from Jackson Labs (Bar Harbor, ME). BMT was performed as previously described.[17,18] Briefly, CBA or B10.BR mice were administered 1,300 cGy total body irradiation in a split dose and injected intravenously with 1×10^7 B10.BR BM cells and 2×10^6 nylon wool-passaged B10.BR splenocytes. Mice were given acidified drinking water and kept in isolator cages after transplantation.

IL-1ra administration. Human IL-1ra was purified as previously described.[13] The allogeneic CBA mice were administered twice daily injections of saline or 3 mg/kg of IL-1ra intraperitoneally (IP) from day 0 through day 10. Syngeneic B10.BR mice were administered only saline injections. The dose of IL-1ra of 3 mg/kg injected twice a day was chosen for this study because a dose of 2 mg/kg injected twice a day had no detectable toxicity and provided complete protection against reactivation arthritis in response to streptococcal cell wall challenge in rats[19] (J. Serwab, manuscript submitted). No toxicities were observed either at this dose and schedule of IL-1ra or at 10 times the dose (20 mg/kg injected twice a day). The administration of IL-1ra at 0.5 mg/kg twice a day gave only 50% protection in the arthritis system. In addition, bioavailability studies in rats have shown that plasma levels of IL-1ra greater than 25 ng/mL can be maintained for at least 8 hours after a 2 mg/kg subcutaneous injection (D. Bloedow, unpublished data). The dose of 3.0 mg/kg injected twice a day was chosen for use in the murine GVHD studies based on the rat data and the different ratio of body surface area to weight in mice. All mice were monitored for potential toxicities with particular reference to lymphohematopoietic reconstitution.

Messenger RNA (mRNA) detection. RNA was isolated by guanidinium thiocyanate/phenol/chloroform extraction, reverse transcribed into cDNA and amplified by polymerase chain reaction (PCR) as described.[20] The PCR protocol consisted of the following conditions: denaturation at 95°C for 30 seconds, annealing at 55°C for 30 seconds, and primer extension at 72°C for 60 seconds. The reactions were performed for 30 cycles. Sense and antisense oligonucleotide primers for TNFα, IL-1α, and β actin yielded PCR products of 310, 300, and 348 bp, respectively, as previously published.[21] PCR products were electrophoresed in a 2% agarose gel and stained with ethidium bromide.

Peripheral blood count determinations. Two hundred microliters

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of heparinized blood was obtained from the retroorbital plexus and complete blood count analysis was obtained by a Technicon H-1 analyzer (Technicon, Tarrytown, NY).

Progenitor assays. Mice were killed by cervical dislocation. BM was harvested, prepared as single cell suspensions, and counted. Progenitors were cultured in duplicate for 7 days using the methyl cellulose technique and the total number of colonies calculated (colony-forming unit granulocyte-macrophage [CFU-GM], $\times 10^{-3}$/2 hind limbs) as described.[22] Hematopoietic stem cells were measured by injection of BM into irradiated (950 cGy) B10.BR mice as described.[23] Spleens were harvested at 12 days (n = 4/5) and the total number of stem cells calculated (CFU-S $\times 10^{-3}$/2 hind limbs).

T-lymphocyte precursor assay. Four weeks after transplantation, mice were killed, and thymus and spleens were prepared as single cell suspensions and counted. Splenocytes were placed in culture for seven days in 96-well plates with the mitogen concanavalin A (3 μg/mL) under limiting dilution conditions as described.[19] Wells were pulsed with ^{3}H-thymidine (New England Nuclear, Boston, MA), harvested, and counted in a beta scintillation counter. Wells scored as positive when the counts per minute were higher than negative controls by at least 3 standard deviations. The frequency of splenic proliferating T-lymphocyte precursors (pPTL) was determined by limiting dilution analysis and the total number of precursors per spleen was calculated by multiplying their frequency by the splenocyte count.

RESULTS

Monokine expression in GVHD skin. We first examined whether the IL-1α gene is transcribed in the skin, a principle target organ, using a murine model of GVHD to minor histocompatibility antigens.[17] As shown in Fig 1, mRNA specific for both TNFα and IL-1α are detectable by PCR amplification of RNA from the skin of animals with GVHD. Neither monokine mRNA is amplified from normal skin. Band fidelity was confirmed by hybridization of the blotted PCR products to the respective γ-P^{32}-labeled internal oligonucleotide probes (data not shown). Negative controls included amplication of RNA without reverse transcriptase that showed no PCR product for β actin. The induction of these inflammatory monokines during GVHD appears to be specific because no PCR product from the mRNA of the lymphokine IL-2 could be detected in the skin of animals with GVHD (data not shown). PCR analysis was used for this study due to the difficulty in discerning TNFα mRNA in the skin of GVHD mice.[24] In addition, we were unable to detect significant differences in TNF protein production from splenocytes of control and GVHD mice using the L929 bioassay (data not shown). Studies are in progress to localize the specific cell populations in the skin that produce both TNFα and IL-1 and the effect of IL-1ra on such production.

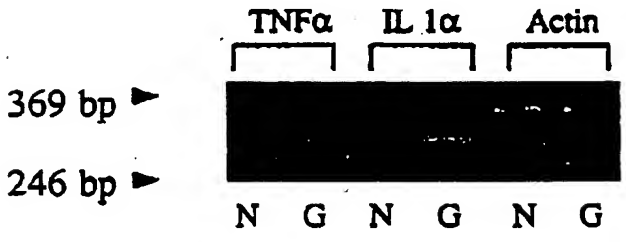

Fig 1. Induction of IL-1 α and TNF α mRNA in GVHD skin. RNA was isolated from epithelial cells of normal CBA mice (N) or transplanted (B10.BR → CBA) mice with GVHD (G), reverse transcribed into cDNA, and amplified by PCR with primers for TNFα, IL-1α, and β actin.

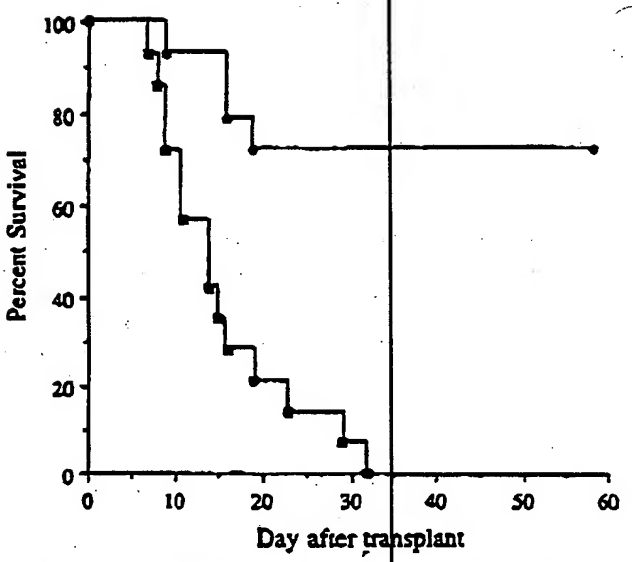

Fig 2. IL-1ra reduces mortality from GVHD. BM and T cells from B10.BR donor mice were injected into lethally irradiated allogeneic CBA recipients. The CBA mice were given twice daily injections of saline (■, n = 14) or 3 mg/kg of IL-1ra IP (●, n = 14) from day 0 through day 10. Survival was significantly improved in the mice receiving IL-1ra (P = .0001, exact log rank test).

Effect of blocking IL-1 on GVHD. To examine further the role of IL-1 in GVHD, we tested the effects of an IL-1 inhibitor, IL-1ra,[13,14] for its effects on systemic GVHD in the same murine BMT model. Transplants of BM and splenic T cells were conducted between donor B10.BR and lethally irradiated recipient CBA mouse strains (B10.BR → CBA) that are H-2 identical but differ at multiple minor histocompatibility loci that are stimuli for GVHD.[17] GVHD induces profound immunosuppression and the mice die within 6 weeks. Transplanted mice received either saline or IL-1ra injections for 10 days after transplantation and were monitored for signs of GVHD and survival (Fig 2). We chose a dose and schedule of IL-1ra treatment based on a regimen that gave complete efficacy and no toxicity in a rat model of arthritic inflammation (see Materials and Methods). The saline-treated (B10.BR → CBA) mice developed evidence of GVHD within 14 to 21 days after BMT and all animals were dead by 40 days after transplantation. By contrast, 80% of the IL-1ra–treated (B10.BR → CBA) mice were alive at day 15 and showed no evidence of GVHD as manifested by weight loss or fur ruffling (data not shown). Survival levelled off at 70% after 60 days after BMT and showed long-term protection by IL-1ra (P = .0001). Syngeneic B10.BR transplant recipients had a 100% survival at 40 days (data not shown). These results argue for an important pathologic role for IL-1 in GVHD.

Effect of blocking GVHD on immune reconstitution. Immunologic dysfunction is another manifestation of GVHD and we wished to see if blocking IL-1 would ameliorate the immunosuppressive effects of GVHD (Table 1).

Table 1. IL-1ra Prevents the Immunologic Suppression of GVHD

Recipient	Injection	n	Thymocyte Counts ($\times 10^{-6}$)	pPTL Total/Spleen ($\times 10^{-6}$)
B10.BR → CBA	Saline	3	0.13	1.14
			(0.1)	(0.48)
B10BR → CBA	IL-1ra	4	2.45	3.52
			(1.49)	(0.99)
B10.BR → B10.BR	Saline	4	4.78	7.02
			(1.09)	(1.83)

CBA (B10.BR → CBA) and B10.BR (B10.BR → B10.BR) mice were transplanted with B10.BR BM and spleen cells as described in Materials and Methods. The frequency of splenic pPTL was determined by limiting dilution analysis and the total number of precursors per spleen was calculated by multiplying their frequency by the splenocyte count. The standard error is in parentheses.

(B10.BR → CBA) control mice treated with saline showed severely diminished thymic cellularity and low numbers of pPTL, as previously reported.[18] By contrast, (B10.BR → CBA) mice receiving IL-1ra had 20 times as many thymocytes and three times as many pPTL per spleen ($P = .03$) when compared with saline-treated (B10.BR → CBA) controls. These data show that IL-1 either directly or indirectly plays an important role in the immunosuppression of GVHD.

Effect of blocking GVHD by IL-1ra on hematopoietic reconstitution. IL-1 is known to be an important factor in hematopoiesis. Exogenous IL-1 protects mice against radiation-induced BM aplasia and enhances BM engraftment in mice receiving T-cell–depleted BMT.[36,37] Recombinant human IL-1ra can block the effect of IL-1 on certain aspects of hematopoiesis in mice.[15] To determine whether blocking the action of endogenous IL-1 might impede engraftment of hematopoietic stem cells, we examined engraftment and hematopoietic reconstitution in IL-1ra–treated animals at 4 weeks after transplantation. Donor B10.BR marrow engrafted completely in CBA mice receiving IL-1ra as determined by the presence of donor B10.BR hemoglobin and thymocytes at 4 and 8 weeks after BMT (data not shown). In addition, IL-1ra had no deleterious effect on peripheral blood counts (Fig 3A). Hematopoietic reconstitution was further quantitated by BM cellularity and the measurement of progenitor cells (CFU-GM) and stem cells (day 12 CFU-S) (Fig 3B). IL-1ra had no deterimental effects on any aspect of hematopoiesis; (B10.BR → CBA) mice receiving IL-1ra had significantly more day 12 CFU-S than the saline-treated (B10.BR → CBA) animals ($P = .05$) and approximately 60% of the day 12 CFU-S of syngeneic (B10.BR → B10.BR) mice. This increase confirms the ability of IL-1ra to block systemic effects of GVHD and to enhance hematopoietic engraftment.

DISCUSSION

Current efforts to prevent GVHD have focused on the inhibition or elimination of T-cell lymphocytes from the donor BM. However, T-cell depletion of donor BM has led to an increase in graft failure, poor immunologic reconstitution, and relapse of primary BM disease.[38] We have shown that IL-1 is an important mediator of GVHD, confirming and extending the observation that exogenous IL-1 appeared to intensify GVHD in another experimental murine model of BMT.[39] The neutralization of endogenous IL-1 by IL-1ra in vivo improved survival from GVHD and reconstitution of cell-mediated immunity without retarding hematopoietic engraftment.

Both TNFα and IL-1 are induced during a variety of inflammatory states and are intimately involved in the pathogenesis of inflammation in such disorders as sepsis and allograft rejection.[7-12] Inhibition of TNFα by anti-TNF antibodies in mice with GVHD has led to highly significant but incomplete inhibition of GVHD mortality.[4] Similarly, in this study, blockade of IL-1 by IL-1ra led to highly significant but not complete inhibition of GVHD mortality. Both cytokines appear to play significant roles in the development and propagation of GVHD. Studies are in progress to determine whether both anti-TNF and anti–IL-1 agents synergize to reduce GVHD mortality. Studies are also in progress to determine the optimal dose and schedule of IL-1ra administration as well as to determine whether IL-1ra will prevent mortality of GVHD that is induced by

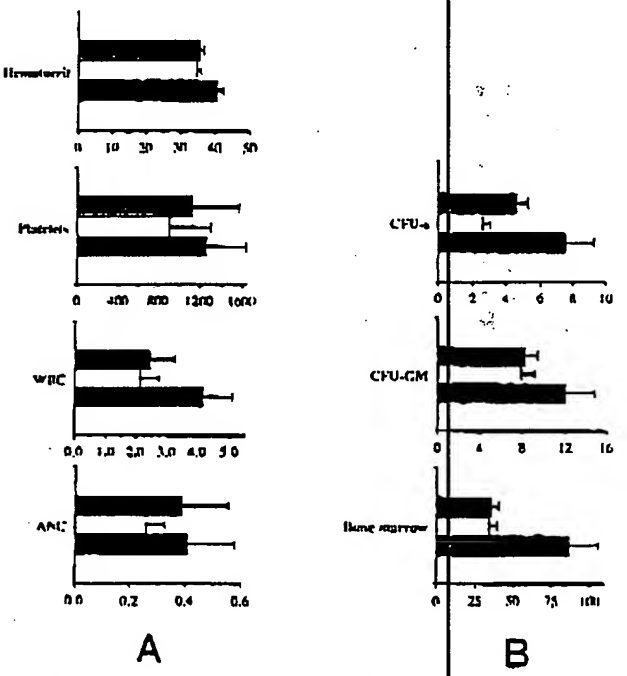

Fig 3. Effect of IL-1ra on hematopoietic reconstitution. (A) Peripheral blood counts from (B10.BR → CBA) recipients treated with IL-1ra (□) were not significantly different from those of (B10.BR → CBA) mice given saline (□) or from (B10.BR → B10.BR) recipients given saline (■). Peripheral blood counts are expressed as: hematocrit (%), platelets ($\times 10^{-9}$/L), white blood count (WBC, $\times 10^{-9}$/L), and absolute neutrophil count ($\times 10^{-9}$/L). (B) BM reconstitution was greater in syngeneic (B10.BR → B10.BR) recipients than in either group of (B10.BR → CBA) recipients. Hematopoietic stem cell numbers (CFU-S day 12) were greater in mice receiving IL-1ra than in saline-treated controls ($P = .05$). Total stem cells are expressed as day 12 CFU-S $\times 10^{-3}$/2 hind limbs. Colony-forming units of granulocyte-monocyte precursors are expressed as CFU-GM $\times 10^{-3}$/2 hind limbs. Nucleated BM cells are expressed as cells $\times 10^{-6}$/2 hind limbs.

MHC differences. Inhibition of IL-1 may permit maintenance of certain critical functions such as immune surveillance while preventing the nonspecific and deleterious effects of systemic inflammation. This drug would be another alternative to T-cell depletion as a means of controlling GVHD. IL-1ra may represent a prototype of a class of cytokine antagonists that will serve not only as a probe for the pathology of a complex disease process, such as GVHD, but also as a new agent for prophylaxis and treatment of clinical GVHD.

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